AMENDMENTS

Amendments to the Specification:

Please replace the paragraph on page 6, lines 16-22, with the following amended paragraph:

The modulator may be a nucleic acid molecule, a polypeptide or protein, a peptide, or a small molecule that affects PPT1 activity, including a peptide mimetic. It is contemplated that any embodiment discussed herein with respect to a peptide may be applied to or with a peptide mimetic. In some embodiments, the modulator is at least one peptide or peptide mimetic that selectively interacts or binds with PPT1. The peptide may contain at most or at least 5 contiguous amino acids from SEQ ID NO:3, for example, the sequence VKIKK (SEQ ID NO:11). In additional embodiments, the peptide is comprised of at most or at least 5 contiguous amino acids from SEQ ID NO:4., such as the sequence YCWLR (SEQ ID NO:12).

Please replace the paragraph on page 7, lines 16-27, with the following amended paragraph:

With respect to any compounds or methods of the invention, it is further contemplated that the modulator will contain or be attached to a lipid component. In some cases, a peptide is attached to a lipid component *e.g*, DAP1 (AcG-palmitoyl diamino propionate-VKIKK (SEQ ID NO:11)) and may be termed "lipopeptide." It is specifically contemplated that the attachment may be achieved though a non-hydrolyzable linkage, such as an amide linkage. In some embodiments a PPT1 modulator that is a peptide is attached to a lipid component. In some cases, the lipid component is a fatty acid, which may be unbranched or not. The lipid component may contain 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or more carbon atoms in length. In some embodiments, the lipid component is 16 carbon atoms in length. In additional embodiments, modulators may be chemically modified. For example, a modulator may be

prepared in an a-ketoamide form, such as with DAP1-ketoamide, where the ketone group is at C15 of the fatty acid. It is contemplated from the crystal structure of PPT1 that a better fit might be a lipid which has a double bond between C4 and C5 of the lipid component. Other structural modifications that introduce a bend or kink into the modulator may be implemented in modulators of the invention. Furthermore, an oxime ether in the lipid is further contemplated as a way of obtaining a more potent inhibitor of PPT1. It is also contemplated to replace the VKIKK (SEQ ID NO:11) peptide sequence with other amino acids and also with non-amino acids such as substituted benzylamines.

Please replace the paragraph on page 8, lines 6-16, with the following amended paragraph:

Other methods of the invention include treating a subject with cancer by administering to the subject a PPT1 modulator in an amount effective to inhibit a cancer cell in the subject, so that a therapeutic benefit is conferred on the subject. As discussed above, the PPT1 modulator may be any modulator disclosed, such as a peptide that selectively interacts with PPT1. Such a peptide may contain at least or at most 5 contiguous amino acids from SEQ ID NO:3, such as the sequence VKIKK (SEQ ID NO:11). Alternatively, such a peptide may contain at least or at most 5 contiguous amino acids from SEQ ID NO:4, for example, the sequence YCWLR (SEQ ID NO:12). The subject may be an mammal, such as a human. Additionally, peptide mimetics of sequences disclosed herein are contemplated as part of the invention. A peptide mimetic of VKIKK (SEQ ID NO:11) or YCWLR (SEQ ID NO:12) are modulators considered for use herein.

Please replace the paragraph on page 11, lines 13-18, with the following amended paragraph:

FIG. 3A and 3B. **FIG. 3A. PPT1 inhibition by a substrate analogue. PPT1 activity was** measured using LA-N-5 cell extract (at pH 4.0 for Po peptide substrate or at pH 7.4 for Gα, GAP43 and rhodopsin peptide substrates) following preincubation with various concentrations of AcG-palmitoyldiaminopropionate-VKIKK SEQ ID NO:11 (DAP1) as described elsewhere in the application. The experiment was repeated twice with similar results. **Fig 3B. Structure of AcG-palmitoyldiaminopropionate-VKIKK SEQ ID NO:11 (DAP1).**

Please replace the paragraph on page 13, lines 26-28, with the following amended paragraph:

FIG. 14. **PPT1 Substrates.** Chemical structures of PPT1 substrates shown. Note that substrate is flurogenic substrate 4-methylumbelliferyl—beta-D-glucosyl-6-thio-palmitate. Glc indicates glucose (SEQ ID NO:11).

Please replace the paragraph on page 17, lines 4-7, with the following amended paragraph:

Fyn Met-[M]Gly-[P]Cys-Val-Gln-Cys-Lys- (SEQ ID NO:13)
Lck Met-[M]Gly-[P]Cys-Val-Cys-Ser-Ser- (SEQ ID NO:14)
Yes Met-[M]Gly-[P]Cys-Ileu-Lys-Ser- (SEQ ID NO:15)
Src Met-[M]Gly-Ser-Ser-Lys-Ser- (SEQ ID NO:16)

Please replace the paragraph on page 28, lines 1-13, with the following amended paragraph:

Peptides that cause a cell to undergo cell death or be susceptible to drugs that induce cell death may be employed. For example, short peptides have been designed that have two functional domains, one a tumor blood vessel "homing" motif such a cyclic CNGRC and the other a programmed cell death-inducing sequence such as the D-enantiomer of

KLAKLAKKLAKLAK (SEQ ID NO:17) connected by a GG bridge (Ellerby *et al.*, 1999). The peptides induced apoptosis in cell lines. The peptides were then tested *in vivo* in nude mice with human MDA-MD-435 breast carcinoma xenografts and found that tumor volume was reduced to 10% of untreated and that 60% were still alive after 100 days compared to none of the untreated. No apparent toxicities were found in mice after 3 months at drug levels of 250 μg/mouse/week. Although the compounds of the present invention appear to bear no relationship to these drugs, the basic principal is the same. Further if there are problems with drug uptake a targetting peptide sequence could be implemented to get the drug into the cell.

Please replace the paragraph on page 43, lines 17-23, with the following amended paragraph:

The spacer arm between the two reactive groups of a cross-linkers may have various length and chemical compositions. A longer spacer arm allows a better flexibility of the conjugate components while some particular components in the bridge (*e.g.*, benzene group) may lend extra stability to the reactive group or an increased resistance of the chemical link to the action of various aspects (*e.g.*, disulfide bond resistant to reducing agents). The use of peptide spacers, such as L-Leu-L-Ala-L-Leu-L-Ala (SEQ ID NO:18), is also contemplated.

Please replace the paragraph on page 76, beginning at line 28, with the following amended paragraph:

Host cells may be derived from prokaryotes or eukaryotes, including yeast cells, insect cells, and mammalian cells, depending upon whether the desired result is replication of the vector or expression of part or all of the vector-encoded nucleic acid sequences. Numerous cell

lines and cultures are available for use as a host cell, and they can be obtained through the American Type Culture Collection (ATCC) on the world wide web at atcc.org, which is an organization that serves as an archive for living cultures and genetic materials ~~(www.atcc.org)~~. An appropriate host can be determined by one of skill in the art based on the vector backbone and the desired result. A plasmid or cosmid, for example, can be introduced into a prokaryote host cell for replication of many vectors. Bacterial cells used as host cells for vector replication and/or expression include DH5α, JM109, and KC8, as well as a number of commercially available bacterial hosts such as SURE® Competent Cells and SOLOPACK™ Gold Cells (STRATAGENE®, La Jolla). Alternatively, bacterial cells such as *E. coli* LE392 could be used as host cells for phage viruses. Appropriate yeast cells include *Saccharomyces cerevisiae*, *Saccharomyces pombe*, and *Pichia pastoris*.

Please replace the paragraph on page 135, lines 6-216, with the following amended paragraph:

[1-^{14}C]palmitoyl CoA (59 mCi/mmol) was purchased from Amersham, Arlington Heights, IL. Synthetic peptides Po (IRYCWLRR (SEQ ID NO:4)), 4Po (RYCW (SEQ ID NO:19)), rhodopsin (VTTLCCGKN (SEQ ID NO:5)), GAP43 (MLCCMRR (SEQ ID NO:6)), Gαs (MGCLGNSK (SEQ ID NO:7)) and H-Ras (GCMSCKCVLS (SEQ ID NO:8)) were purchased from Research Genetics, Huntsville, AL. The peptide sequence selected was based on the palmitoylation motif of endogenous proteins (Bizzozero, 1997). The inhibitor peptide (AcG-palmitoyl diamino propionate-VKIKK (SEQ ID NO:11)) and its base peptide (AcGCVKIKK (SEQ ID NO:20) was synthesized as descibed below. Boc-Dap(Fmoc) was from Bachem Bioscience (King of Prussia, PA), hydrogen fluoride was from Matheson Gas (Cucamonga,CA)

and amino acids from Midwest Biotech (IN). Tfx-50 transfection reagent was from Promega (Madison, WI) and solvents were ACS grade from Fisher Scientific, Pittsburgh, PA.

Please replace the paragraph on page 135, lines 19-21, with the following amended paragraph:

2. Peptide inhibitor synthesis

The peptides AcGCVKIKK (SEQ ID NO:20) and AcG (palmitoyl diaminoproprionate-VKIKK (SEQ ID NO:11) were synthesized by solid phase peptide synthesis using in situ neutralization cycles for Boc chemistry (Schnolzer et al., 1992).

Please replace the paragraph on page 136, lines 15-26, with the following amended paragraph:

4. Preparation of [^{14}C]palmitoylated substrates

Palmitoylation of peptides was performed as described previously with slight modifications (Cho and Dawson, 1998; Bharadwaj and Bizzozero, 1995). Fifty μg of each synthetic peptide (IRYCWLRR (SEQ ID NO:4) (Po), RYCW (SEQ ID NO:19) (4Po), VTTLCCGKN (SEQ ID NO:5) (rhodopsin), MLCCMRR (SEQ ID NO:6) (GAP43), MGCLGNSK (SEQ ID NO:7) (Gα) or GCMSCKCVLS (SEQ ID NO:8) (H-Ras)) was incubated with approximately 2 nmol of [1-^{14}C]palmitoyl CoA (59 mCi/mmol) in 0.1 M 3-[N-morpholino]propanesulfonic acid (MOPS) buffer (pH 7.4) containing 1 mM DTT and 0.1% Triton X-100. The reaction was stopped by immediately placing the tube at - 20° C. Palmitoyl peptides were separated by HPTLC using n-butanol/pyridine/acetic acid/water (45:30:9:36, v/v). After developing the autoradiogram, the radioactive spot corresponding to the palmitoyl peptide was extracted and reconstituted in 25 % methanol except the GCMSCKCVLS (SEQ ID NO:8) reaction mixture, which was unable to be resolved by HPTLC.

Please replace the paragraph on page 139, lines 8-24, with the following amended paragraph:

F. Synthesis of a specific inhibitor of PPT

Based on the capability of PPT1 to depalmitoylate peptides where palmitate is linked to a cysteine residue via a thioester bond, that a non-hydrolyzable palmitoylated peptide analogue would block PPT activity was investigated. The peptide sequence, AcGCVKIKK (SEQ ID NO:20) (a palmitoylation site in K-Ras), was modified by substituting the SH group of cysteine with NH_2 to generate diamino propionic acid, which was then acylated with palmitate (AcG-palmitoyl diamino propionate-VKIKK (SEQ ID NO:11). This results in a CONH amide linkage instead of a COS thioester linkage. PPT activity was measured using indicated substrates in a standard way as described above, except that the cell extract was preincubated with various concentrations of the analogue substrate for 15 min at 37°C prior to the addition of radiolabeled substrate. As shown in FIG. 3, the presence of the amide substrate efficiently inhibited PPT1 activity. The inhibition was more potent towards Po, rhodopsin and GAP-43 peptides than towards Gα peptide. In another set of experiments, the unmodified peptide itself (AcGCVKIKK (SEQ ID NO:20) was used as a control to exclude the possibility of a nonspecific effect of the sequence on PPT activity. Preincubation of cell extract with AcGCVKIKK (SEQ ID NO:20 itself did not affect PPT activity toward any of the substrates tested.

Please replace the paragraph on page 140, lines 22-28, with the following amended paragraph:

2. Preparation of hPPT1 cDNA and plasmid construction

cDNA for human PPT1 gene was prepared by reverse-transcription polymerase chain reaction using RNA isolated from LA-N-5 cells. The first strand cDNA was amplified with a forward primer:

5' TCTAGGTACCAAGATGGCGTCGCCCGGCTGCCTGT 3' (SEQ ID NO:9)

and a reverse primer:

5' ACGGTCTAGATCATCCAAGGAATGGTATGATGTGGGCA 3' (SEQ ID NO:10)

Please replace the paragraph on page 141, lines 16-25, with the following amended paragraph:

4. PPT1 assay

PPT1 activity was measured as described previously (Cho and Dawson, 1998). In brief, the cell sonicate was incubated in the assay mixture containing 50 mM sodium citrate (pH 4.0) and IRY([^{14}C]palmitoylated)CWLRR (SEQ ID NO:4) octapeptide (2,000-4,000 cpm) for 20 min at 37 °C. For the pH profile of PPT1 activity, 50 mM sodium citrate (pH 5 or 6) or 50 mM Tris-HCl (pH 7.4) was used with other conditions remaining the same. After addition of 1 ml of chloroform/methanol/2N HCl (2:1:0.06, by volume) and centrifugation, the organic phase was dried, reconstituted sample analyzed by HPTLC using n-butanol/pyridine/acetic acid/water (45:30:9:36, by volume) and the radioactive spot corresponding to [^{14}C] palmitate was counted.

Please replace the paragraph on page 149, lines 14-21, with the following amended paragraph:

4. PPT1 assay

PPT1 activity was measured as described above. In brief, the cell sonicate was incubated in the assay mixture (50 mM sodium citrate (pH 4.0) for [^{14}C]palmitoylated IRYCWLRR (SEQ ID NO:4) Po peptide substrate (3,000-4,000 cpm) or 50 mM Tris (pH 7.4) for [^{14}C]palmitoylated

MLCCMRR (SEQ ID NO:6) GAP43 peptide substrate) for 20 min at 37 °C. After organic extraction, samples were analyzed by HPTLC using n-butanol/pyridine/acetic acid/water (45:30:9:36, by volume) and the radioactive spot corresponding to [^{14}C] palmitate was counted.

Please replace the paragraph on page 152, lines 15-23, with the following amended paragraph:

E. PPT1 Antisense Treatment Reduces PPT1 Activity

To provide further evidence that treatment with AS-PPT results in inhibition of PPT1, PPT1 enzyme activity was measured directly by an *in vitro* assay (Cho & Dawson, 1998), using cell extracts from either control or AS-PPT1- treated cells. Addition of AS-PPT1 resulted in a 12 %- 18 % reduction of PPT1 activity compared to control, when either [^{14}C]-palmitoyl-IRYCWLRR (SEQ ID NO:4) (Po) or [^{14}C]- palmitoyl- MLCCMRR (SEQ ID NO:6) (GAP43) peptide were used as the substrate (FIG. 9). The reduction in PPT1 activity compares well to the expected cell transient transfection efficiency of 10-20 %.

Please replace the paragraph on page 155, beginning at line 6, with the following amended paragraph:

A tumor cell line, such as LAN-5, a human neuroblastoma cell line will be employed. Such cell lines may be obtained through ATCC on the world wide web at atcc.org ~~(www.atcc.org)~~. Tumor cells will be injected into nude mice. PPT1 inhibitors will be synthesized or purchased. In addition the mice may be given a chemotherapeutic agents, such as etoposide and daunorubicin.

Please replace the paragraph on page 158, lines 19-25, with the following amended paragraph:

Keto amide- and keto ester-based unhibitors of proteases have been shown to have greater specificity than traditional fluorinated ketone-type inhibitors in these systems (Ogilvie *et al.*, 1997; Slee *et al.*, 1995). Recently, α-keto amide triglyceride analogs have been synthesized as inhibitors of *Staphylococcus hyicus* lipase (Simmons *et al.*, 1999) and pancreatic lipase (Chiou *et al.*, 2000). The fully protected peptide AcG-Dap((+/-)α-hydroxyhexadecanoyl)-VKIKK (SEQ ID NO:11).amide (DAP-KA) was synthesized by solid phase peptide synthesis in good (mg) yield (FIG. 14).